REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. AGENCY USE ONLY *(Leave blank)*	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	October 1, 2002	Final report 4/1/01 – 3/31/02

4. TITLE AND SUBTITLE
Instrumentation for the interfacial analysis for biosensor microsystems containing genetically engineered proteins.

5. FUNDING NUMBERS
N00014-01-1-0559

6. AUTHOR(S)
Homme W. Hellinga

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Department of Biochemistry
Duke University Medical Center
Durham, NC 27710

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
Office of Naval Research
800 North Quincy Street
Arlington, VA 22217-5000

10. SPONSORING / MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION / AVAILABILITY STATEMENT
Distribution unlimited

12b. DISTRIBUTION CODE

13. ABSTRACT *(Maximum 200 words)*

ABSTRACT

The combined use of an atomic force microscope and ellipsometer allows determination of the structure of immobilized protein layers (thickness, orientation, surface concentration, homogeneity), kinetics of layer formation, transport properties of ligand between bulk and protein layer, and stability of the protein layer as compared to solution stability. We have characterized monolayers formed by covalent attachment of E. coli periplasmic binding proteins to glass and other substrates, and found that the orientation of these proteins can be controlled precisely, and that the monolayers retain their ability to respond to analytes with a ligand-mediated hinge-bending conformational change.

14. SUBJECT TERMS
Biosensors; protein engineering; Microsystems; nanotechnology

15. NUMBER OF PAGES
3

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	UL

FINAL REPORT

GRANT #: N00014-01-1-0559

PRINCIPAL INVESTIGATOR: Dr. Homme W. Hellinga

INSTITUTION: Duke University Medical Center

GRANT TITLE: Instrumentation for the interfacial analysis of biosensor Microsystems containing genetically engineered proteins

AWARD PERIOD: 1 April 2001 to 30 March 2002

OBJECTIVE: Purchase of instrumentation for the analysis of biomolecular interfaces constructed to integrate engineered proteins into detector devices.

APPROACH: The combined use of an atomic force microscope and ellipsometer allows determination of the structure of immobilized protein layers (thickness, orientation, surface concentration, homogeneity), kinetics of layer formation, transport properties of ligand between bulk and protein layer, stability of the protein layer as compared to solution stability, and the stability of attachment chemistries.

ACCOMPLISHMENTS: We have successfully used the atomic force microscopy (AFM) and ellipsometer to characterize monolayers of biosensor proteins attached to a silicon substrate.

Over the last few years, we have explored the use of genetically engineered, stable, soluble bacterial receptor proteins for the systematic development of novel biosensors. In order to develop reagentless, integrated biosensors incorporating these proteins, three sensor components have to be engineered at the molecular level: a) signal transduction, b) molecular recognition, and c) nanostructured interfaces that integrate the receptors into detectors. We have demonstrated the feasibility of engineering the first two components. We have been able to show that we can drastically alter the ligand-binding specificities of these proteins, using computational design techniques to predict the necessary mutations. In this manner, we have created sensors for trinitrotoluene, and a simulant for the nerve agent soman, among others. Less well developed are the methods for integrating these engineered proteins into sensor devices by attachment to optical fibers, for instance. More recently, we have developed new chemistries that permit multiple cysteines to be modified independently, allowing one cysteine to be used for introducing reporter functionalities, and the other for orientation-specific covalent coupling to a glass substrate. The instrumentation purchased by this grant has been invaluable in characterizing the immobilized proteins.

The AFM and ellipsometer have allowed us to characterize geometrical patterns and the thickness of the immobilized protein films respectively. We found that the dual cysteine modifications work well. One of the cysteines can be modified with a fluorescent (or redox) reporter group, leaving the other available for covalent attachment

to a solid support. The proteins were attached to glass slide modified with thiol-reactive maleimide groups. We found that if the maleimides were patterned using UV light to selective oxidize them, the proteins are attached only to those areas that were protected by the mask, and available for reacting with the thiol (AFM measurements). The edges of the protein monolayers were "sharp", indicating good specificity of reactivity. Ellipsometry revealed that the protein was indeed laid down as a monolayer.

PBPs are ellipsoid, with an approximately two-fold difference in the length of the axes. The orientation of the monolayer is determined by the position of the thiol attachment point on the surface of the protein. We designed two thiol mutations that are predicted to orient the protein with the longest axis perpendicular or the shortest axis perpendicular to the substratum, respectively. Ellipsometry revealed that for the long axis mutation, we obtained a 75Å layer, and for the short axis a 35Å layer; thicknesses that correspond within a few Ångstroms of the crystallographically measured protein thickness. Furthermore, addition of ligand resulted in a change in layer thickness, corresponding to a change in the conformation of the protein, decreasing the thickness in the case of the long axis and increasing in the case of the short axis. These results show that the orientation of the protein in the monolayer can be controlled, and that the proteins retain their conformational response upon binding of ligands.

Currently we are using the ellipsometer to measure the stability of the protein monolayer, by determining the change in thickness as a function of urea addition. This will provide us with new insights into the influence of immobilization on protein stability.

This work is being prepared for publication, and a patent protecting these concepts will be applied for.

CONCLUSIONS: Engineered periplasmic binding proteins can be put down in an orientation-specific manner on glass substrates and retain their ability to bind analyte and transduce signal.

SIGNIFICANCE: Engineered periplasmic binding proteins are strong candidates for the development of biosensors. Integration of such proteins into devices is an important aspect of their utility.